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(54) Title: TARGETABLE VECTOR PARTICLES (57) Abstract A vector particle (e.g., a retroviral vector particle) containing a chimeric envelope includes a receptor binding region that binds to a receptor of a target cell. The receptor of the target cell is other than the amphotropic cell receptor. The receptor binding region may be a receptor binding region of a human virus. A portion of the envelope gene may be deleted and the deleted portion is replaced with another receptor binding region or ligand. Such vector particles are targetable to a desired target cell or tissue, and may be administered directly to the desired target cell or tissue as part of a gene therapy procedure, or administered directly into the patient.		

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TARGETABLE VECTOR PARTICLES

This invention relates to "targetable" vector particles. More particularly, this invention relates to vector particles which include a receptor binding region that binds to a receptor of a target cell of a human or non-human animal.

Vector particles are useful agents for introducing gene(s) or DNA (RNA) into a cell, such as a eukaryotic cell. The gene(s) is controlled by an appropriate promoter. Examples of vectors which may be employed to generate vector particles include prokaryotic vectors, such as bacterial vectors; eukaryotic vectors, including fungal vectors such as yeast vectors; and viral vectors such as DNA virus vectors, RNA virus vectors, and retroviral vectors. Retroviral vectors which have been employed for generating vector particles for introducing genes or DNA (RNA) into a cell include Moloney Murine Leukemia Virus, spleen necrosis virus, and vectors derived from retroviruses such as Rous Sarcoma Virus and Harvey Sarcoma Virus. The term "introducing" as used herein encompasses a variety of methods of transferring genes or DNA (RNA) into a cell, such methods including transformation, transduction, transfection, and infection.

Vector particles have been used for introducing DNA (RNA) into cells for gene therapy purposes. In general, such a procedure involves obtaining cells from a patient and using a vector particle to introduce desired DNA (RNA) into the cells and

then providing the patient with the engineered cells for a therapeutic purpose. It would be desirable to provide alternative procedures for gene therapy. Such an alternative procedure would involve genetically engineering cells in vivo. In such a procedure, a vector particle which includes the desired DNA (RNA) would be administered directly to the target cells of a patient in vivo.

It is therefore an object of the present invention to provide gene therapy by introduction of a vector particle, such as, for example, a retroviral vector particle, directly into a desired target cell of a patient.

In accordance with an aspect of the present invention, there is provided a retroviral vector particle which includes a receptor binding region or ligand that binds to a receptor of a target cell. The receptor of the target cell is a receptor other than the amphotropic cell receptor.

Retroviruses have an envelope protein which contains a receptor binding region. Applicants have found that retroviruses can be made "targetable" to a specific type of cell if the receptor binding region of the retrovirus, which may be amphotropic, ecotropic, or xenotropic, among other types, is modified such that the receptor binding region of the envelope protein includes a receptor binding region which binds to a receptor of a target cell. For example, at least a portion of the receptor binding region of the envelope protein of the retrovirus is deleted and replaced with a receptor binding region or a ligand which binds to a receptor of a target cell. Thus, there is provided a retroviral vector wherein at least a portion of the DNA (RNA) which encodes the receptor binding region of the envelope protein of the retrovirus has been deleted and replaced with DNA (RNA) encoding a receptor binding region or a ligand which binds to a receptor of a target cell.

In one embodiment, the retrovirus is a murine leukemia virus.

The envelope of murine leukemia viruses includes a protein known as gp70. Such viruses can be made "targetable" to a specific type of cell if a portion of the gp70 protein is deleted and replaced with a receptor binding region or a ligand which binds to a receptor of a target cell. Thus, in a preferred embodiment, there is provided a retroviral vector wherein a portion, but not all, of the DNA (RNA) encoding gp70 protein has been deleted and replaced with DNA (RNA) encoding a receptor binding region or a ligand which binds to a receptor of a target cell.

In general, gp70 protein includes the following regions: (i) the secretory signal or "leader" sequence; (ii) the receptor binding domain; (iii) the hinge region; and (iv) the body portion. Preferably, at least a portion of the DNA (RNA) encoding the receptor binding domain of gp70 protein is deleted and replaced with DNA (RNA) encoding a receptor binding region or a ligand which binds to a receptor of a target cell. More preferably, DNA (RNA) encoding the entire receptor binding domain of gp70 protein is deleted and replaced with DNA (RNA) encoding a receptor binding region or a ligand which binds to a receptor of a target cell. In another embodiment, DNA (RNA) encoding the entire receptor binding domain of gp70 protein, plus all or a portion of the DNA (RNA) encoding the hinge region of gp70 protein is deleted and replaced with DNA (RNA) encoding a receptor binding region or a ligand of a target cell.

The gp70 protein may be derived from an ecotropic murine leukemia virus, a xenotropic murine leukemia virus, or an amphotropic murine leukemia virus. Ecotropic gp70 (or eco gp70) (SEQ ID NO:1) is a protein having 469 amino acids, and is encoded by (SEQ ID:2). Amino acid residues 1-33 constitute the leader sequence; amino acid residues 34-263 constitute the receptor binding domain; amino acid residues 264-312 constitute the hinge region; and amino acid residues 313-469 constitute the body portion. Preferably, DNA (RNA) encoding at least a portion of

the receptor binding region is removed and replaced with DNA (RNA) encoding a receptor binding region or a ligand which binds to a receptor of a target cell. More preferably, DNA (RNA) encoding some or all of amino acid residues 34 to 263 (i.e., the receptor binding domain) is removed and replaced with DNA (RNA) encoding a receptor binding region or a ligand which binds to a receptor of a target cell.

Xenotropic gp70 (or xeno gp70) (SEQ ID NO:3) has 443 amino acid residues and is encoded by (SEQ ID NO:4). Amino acid residues 1-30 constitute the leader sequence; amino acid residues 31-232 constitute the receptor binding domain; amino acid residues 233-286 constitute the hinge region; and amino acid residues 287-443 constitute the body portion. Preferably, DNA (RNA) encoding at least a portion of the receptor binding region is removed and replaced with DNA (RNA) encoding a receptor binding region or a ligand which binds to a receptor of a target cell. More preferably, DNA (RNA) encoding some or all of amino acid residues 31 to 232 is removed and replaced with DNA (RNA) encoding a receptor binding region or a ligand which binds to a receptor of a target cell.

Target cells to which the retroviral vector particle may bind include, but are not limited to, liver cells, T-cells, lymphocytes, endothelial cells, T4 helper cells, and macrophages. In one embodiment, the retroviral vector particle binds to a liver cell, and in particular to hepatocytes. To enable such binding, the retroviral vector particle contains a chimeric protein encoded by DNA (RNA) in which at least a portion of the DNA (RNA) encoding the receptor binding domain of gp70 protein is removed and is replaced with DNA (RNA) which encodes a protein which binds to an asialoglycoprotein receptor (or ASG-R) of hepatocytes.

Proteins which bind to the asialoglycoprotein receptor of liver cells include, but are not limited to, asialoglycoproteins such as, for example, alpha-1-acid glycoprotein (AGP), also known

as orosomucoid, and asialofetuin. AGP is a natural high-affinity ligand for ASG-R. The asialoglycoprotein receptor, or ASG-R, is expressed only by hepatocytes. The receptor is present at about 3×10^5 copies per cell, and such receptors have a high affinity for asialoglycoproteins such as AGP. Thus, the engineering of retroviral vector particles to contain asialoglycoprotein in place of the natural receptor binding domain of gp70 generates retroviral vector particles which bind to the asialoglycoprotein receptor of hepatocytes, which provides for an efficient means of transferring genes of interest to liver cells.

Cell lines which generate retroviral vector particles that are capable of targeting the hepatocyte's asialoglycoprotein receptor without the removal of the particle's terminal sialic acid groups by neuraminidase treatment, can be developed by selection with the cytotoxic lectin, wheat germ agglutinin (WGA). Cell lines which express the retroviral proteins gag and pol become retroviral vector packaging cell lines after they are transfected with the plasmids encoding chimeric envelope genes. These cell lines express the corresponding chimeric gp 70 glycoproteins. Upon exposure to successively higher concentrations of WGA, the outgrowth of cells which synthesize glycoproteins that lack terminal sialic acid groups, is favored. (Stanley, et al., Somatic Cell Genetics, Vol. 3, pgs. 391-405 (1977)). This selection permits the isolation of cells which synthesize oligosaccharides terminating in galactosyl sugar groups. Such cells will allow the construction of packaging cell lines that are capable of generating retroviral vector particles which target the asialoglycoprotein receptor. It is also possible to select subpopulations of packaging cells which have other distinct glycotypes, such cells yielding viral vectors that potentially are capable of targeting cells other than hepatocytes. Macrophages, for example, express unique, high-mannose receptors. The PHA-resistant subpopulation will have N-linked oligosaccharides which terminate in high-mannose

groups (Stanley, et al., In Vitro, Vol. 12, pgs. 208-215 (1976)). Therefore, such a cell population will be capable of producing viral vector particles capable of targeting macrophages via this receptor. Cells with mutant glycotypes which synthesize other novel oligosaccharides after selection with other cytotoxic lectins may also prove to be useful in targeting vector particles to other cell types such as lymphocytes or endothelial cells.

In another embodiment, the receptor binding region is a receptor binding region of a human virus. In one embodiment, the receptor binding region of a human virus is a hepatitis B virus surface protein binding region, and the target cell is a liver cell.

In another embodiment, the receptor binding region of a human virus is the gp46 protein of HTLV-I virus, and the target cell is a T-cell.

In yet another embodiment, the receptor binding region of a human virus is the HIV gp120 CD4 binding region, and the target cell is a T4 helper cell.

In one embodiment, the retroviral vector may be of the LN series of vectors, as described in Bender, et al., J. Virol., Vol. 61, pgs. 1639-1649 (1987), and Miller, et al., Biotechniques, Vol. 7, pgs. 98-990 (1989).

In another embodiment, the retroviral vector includes a multiple restriction enzyme site, or multiple cloning site. The multiple cloning site includes at least four cloning, or restriction enzyme sites, wherein at least two of the sites have an average frequency of appearance in eukaryotic genes of less than once in 10,000 base pairs; i.e., the restriction product has an average size of at least 10,000 base pairs.

In general, such restriction sites, also sometimes hereinafter referred to as "rare" sites, which have an average frequency of appearance in eukaryotic genes of less than once in 10,000 base pairs, contain a CG doublet within their recognition sequence, such doublet appearing particularly infrequently in the

mammalian genome. Another measure of rarity or scarcity of a restriction enzyme site in mammals is its representation in mammalian viruses, such as SV40. In general, an enzyme whose recognition sequence is absent in SV40 may be a candidate for being a "rare" mammalian cutter.

Examples of restriction enzyme sites having an average frequency of appearance in eukaryotic genes of less than once in 10,000 base pairs include, but are not limited to the NotI, SnaBI, SalI, XhoI, ClaI, SacI, EagI, and SmaI sites. Preferred cloning sites are selected from the group consisting of NotI, SnaBI, SalI, and XhoI.

Preferably, the multiple cloning site has a length no greater than about 70 base pairs, and preferably no greater than about 60 base pairs. In general, the multiple restriction enzyme site, or multiple cloning site is located between the 5' LTR and 3' LTR of the retroviral vector. The 5' end of the multiple cloning site is no greater than about 895 base pairs from the 3' end of the 5' LTR, preferably at least about 375 base pairs from the 3' end of the 5' LTR. The 3' end of the multiple cloning site is no greater than about 40 base pairs from the 5' end of the 3' LTR, and preferably at least 11 base pairs from the 5' end of the 3' LTR.

Such vectors may be engineered from existing retroviral vectors through genetic engineering techniques known in the art such that the retroviral vector includes at least four cloning sites wherein at least two of the cloning sites are selected from the group consisting of the NotI, SnaBI, SalI, and XhoI cloning sites. In a preferred embodiment, the retroviral vector includes each of the NotI, SnaBI, SalI, and XhoI cloning sites.

Such a retroviral vector may serve as part of a cloning system for the transfer of genes to such retroviral vector. Thus, there may be provided a cloning system for the manipulation of genes in a retroviral vector which includes a retroviral vector including a multiple cloning site of the type hereinabove

described, and a shuttle cloning vector which includes at least two cloning sites which are compatible with at least two cloning sites selected from the group consisting of NotI, SnaBI, SalI, and XhoI located on the retroviral vector. The shuttle cloning vector also includes at least one desired gene which is capable of being transferred from said shuttle cloning vector to said retroviral vector.

The shuttle cloning vector may be constructed from a basic "backbone" vector or fragment to which are ligated one or more linkers which include cloning or restriction enzyme recognition sites. Included in the cloning sites are the compatible, or complementary cloning sites hereinabove described. Genes and/or promoters having ends corresponding to the restriction sites of the shuttle vector may be ligated into the shuttle vector through techniques known in the art.

The shuttle cloning vector may be employed to amplify DNA sequences in prokaryotic systems. The shuttle cloning vector may be prepared from plasmids generally used in prokaryotic systems and in particular in bacteria. Thus, for example, the shuttle cloning vector may be derived from plasmids such as pBR322; pUC18; etc.

Such retroviral vectors are transfected or transduced into a packaging cell line, whereby there are generated infectious vector particles which include the retroviral vector. In general, the vector is transfected into the packaging cell line along with a packaging defective helper virus which includes genes encoding the gag and pol, and the env proteins of the virus. Representative examples of packaging cell lines include, but are not limited to, the PE501 and PA317 cell lines disclosed in Miller, et al., Biotechniques, Vol. 7 pgs. 980-990 (1989).

The vector particles generated from the packaging cell line, which are also engineered with a protein containing a receptor binding region that binds to a receptor of a target cell, said receptor being other than the amphotropic cell receptor, are

targetable, whereby the receptor binding region enables the vector particles to bind to a target cell. The retroviral vector particles thus may be directly administered to a desired target cell ex vivo, and such cells may then be administered to a patient as part of a gene therapy procedure.

Although the vector particles may be administered directly to a target cell, the vector particles may be engineered such that the vector particles are "injectable" as well as targetable; i.e., the vector particles are resistant to inactivation by human serum, and thus the targetable vector particles may be administered to a patient by intravenous injection, and travel directly to a desired target cell or tissue without being inactivated by human serum.

The envelope of retroviruses also includes a protein known as p15E, and Applicants have found that retroviruses are susceptible to inactivation by human serum as a result of the action of complement protein(s) present in serum on the p15E protein portion of the retrovirus. Applicants have further found that such retroviruses can be made resistant to inactivation by human serum by mutating such p15E protein.

In one embodiment, therefore, the retroviral vector is engineered such that a portion of the DNA (RNA) encoding p15E protein (shown in the accompanying sequence listing as SEQ ID NO:7), has been mutated to render the vector particle resistant to inactivation by human serum; i.e., at least one amino acid but not all of the amino acids of the p15E protein has been changed, or mutated.

p15E protein is a viral protein having 196 amino acid residues. In viruses, sometimes all 196 amino acid residues are present, and in other viruses, amino acid residues 181 to 196 (known as the "r" peptide), are not present, and the resulting protein is the "mature" form of p15E known as p12E. Thus, viruses can contain both the p15E and p12E proteins. p15E protein is anchored in the viral membrane such that amino acid

residues residues 1 to 134 are present on the outside of the virus. Although this embodiment of the present invention is not to be limited to any of the following reasoning, Applicants believe complement proteins may bind to this region whereby such binding leads to inactivation and/or lysis of the retrovirus. In particular, the p15E protein includes two regions, amino acid residues 39 to 61 (sometimes hereinafter referred to as region 1), and amino acid residues 101 to 123 (sometimes hereinafter referred to as region 2), which Applicants believe have an external location in the three-dimensional structure of the p15E protein; i.e., such regions are directly exposed to human serum. Region 2 is a highly conserved region in many retroviruses, even though the amino acid sequences of this region are not identical in all retroviruses. Such regions are complement binding regions. Examples of complement proteins which may bind to the complement binding regions are C1S and C1Q, which bind to regions 1 and 2.

In order to inactivate the retrovirus, complement proteins bind to both region 1 and region 2. Thus, in a preferred embodiment, at least one portion of DNA encoding a complement binding region of p15E protein has been mutated. Such a mutation results in a change of at least one amino acid residue of a complement binding region of p15E protein. The change in at least one amino acid residue of a complement binding region of p15E protein prevents binding of a complement protein to the complement binding region, thereby preventing complement inactivation of the retrovirus. In one embodiment, at least one amino acid residue in both complement binding regions of p15E protein is changed, whereas in another embodiment, at least one amino acid residue in one of the complement binding regions is changed.

It is to be understood, however, that the entire DNA sequence encoding p15E protein cannot be mutated because such a change renders the vectors unsuitable for in vivo use.

In one embodiment, the mutation of DNA (RNA) encoding p15E protein may be effected by deleting a portion of the p15E gene, and replacing the deleted portion of the p15E gene, with fragment(s) or portion(s) of a gene encoding another viral protein. In one embodiment, one portion of DNA encoding the p15E protein is replaced with a fragment of the gene encoding the p21 protein, which is an HTLV-I transmembrane protein. HTLV-I virus has been found to be resistant to binding by complement proteins and thus HTLV-I is resistant to inactivation by human serum (Hoshino, et al., Nature, Vol. 310, pgs. 324-325 (1984)). Thus, in one embodiment, there is also provided a retroviral vector particle wherein a portion of the p15E protein has been deleted and replaced with a portion of another viral protein, such as a portion of the p21 protein.

p21 protein (as shown in the accompanying sequence listing as SEQ ID NO:8) is a protein having 176 amino acid residues, and which, in relation to p15E, has significant amino acid sequence homology. In one embodiment, at least amino acid residues 39 to 61, and 101 to 123 are deleted from p15E protein, and replaced with amino acid residues 34 to 56 and 96 to 118 of p21 protein. In one alternative, at least amino acid residues 39 to 123 of p15E protein are deleted and replaced with amino acid residues 34 to 118 of p21 protein.

In another embodiment, amino acid residues 39 to 69 of p15E protein are deleted and replaced with amino acid residues 34 to 64 of p21 protein, and amino acid residues 96 to 123 of p15E protein are deleted and replaced with amino acid residues 91 to 118 of p21 protein.

Vector particles generated from such packaging lines, therefore, are "targetable" and "injectable," whereby such vector particles, upon administration to a patient, travel directly to a desired target cell or tissue.

The targetable vector particles are useful for the introduction of desired heterologous genes into target cells ex

vivo. Such cells may then be administered to a patient as a gene therapy procedure, whereas vector particles which are targetable and injectable may be administered in vivo to the patient, whereby the vector particles travel directly to a desired target cell.

Thus, preferably, the vectors or vector particles of the present invention further include at least one heterologous gene. Heterologous or foreign genes which may be placed into the vector or vector particles include, but are not limited to, genes which encode cytokines or cellular growth factors, such as lymphokines, which are growth factors for lymphocytes. Other examples of foreign genes include, but are not limited to, genes encoding Factor VIII, Factor IX, tumor necrosis factors (TNF's), ADA, ApoE, ApoC, and Protein C.

The vectors of the present invention include one or more promoters. Suitable promoters which may be employed include, but are not limited to, the retroviral LTR; the SV40 promoter; and the human cytomegalovirus (CMV) promoter described in Miller, et al., Biotechniques, Vol. 7, No. 9, pgs 980-990 (1989), or any other promoter (e.g., cellular promoters such as eukaryotic cellular promoters including, but not limited to, the histone, pol III, and B-actin promoters). Other viral promoters which may be employed include, but are not limited to, adenovirus promoters, TK promoters, and B19 parvovirus promoters. The selection of a suitable promoter will be apparent to those skilled in the art from the teachings contained herein.

The vectors of the present invention may contain regulatory elements, where necessary to ensure tissue specific expression of the desired heterologous gene(s), and/or to regulate expression of the heterologous gene(s) in response to cellular or metabolic signals.

Although the invention has been described with respect to retroviral vector particles, other viral vector particles (such as, for example, adenovirus, adeno-associated virus, and Herpes

Simplex virus particles), or synthetic particles may be constructed such that the vector particles include a receptor binding region that binds to a receptor of a target cell, wherein the receptor of a human target cell is other than the amphotropic cell receptor. Such vector particles are suitable for in vivo administration to a desired target cell.

Advantages of the present invention include the ability to provide vector particles which may be administered directly to a desired target cell or tissues, whereby desired genes are delivered to the target cell or tissue, whereby the target cell or tissue may produce the proteins expressed by such genes.

This invention will now be described with respect to the following examples; however, the scope of the present invention is not intended to be limited thereby.

Example 1

Plasmid pCee (Figure 1), which contains the ecotropic murine leukemia virus gp70 and p15E genes under the control of a CMV promoter, was cut with AccI, and an AccI fragment encoding amino acid residues 1-312 of the eco gp70 protein was removed. Cloned into the AccI site was a PCR fragment containing the eco gp70 secretion signal (or leader, which includes amino acid residues 1-33 of eco gp70), followed by mature rabbit alpha-1 acid glycoprotein (amino acid residues 19-201) (Ray, et al., Biochemical and Biophysical Research Communications, Vol. 178, No. 2, pgs. 507-513 (1991)). The amino acid sequence of rabbit alpha-1 acid glycoprotein is shown in (SEQ ID NO:5), and the DNA sequence encoding therefor is shown in (SEQ ID NO:6). The resulting plasmid pAGP-1 (Figure 2) contains the eco gp70 leader sequence (amino acid residues 1-33 of eco gp70), a sequence encoding the mature rabbit alpha-1 acid glycoprotein (amino acid residues 19-201), and a sequence encoding amino acid residues 313 to 469 of eco gp70.

Example 2

Plasmid pCee was cut with SalI and PflMI, and a SalI-PflMI fragment encoding amino acid residues 1-262 of eco gp70 was removed. Cloned into this site was a PCR generated SalI-PflMI fragment containing the eco gp70 leader sequence and the sequence encoding mature rabbit alpha-1 acid glycoprotein. The resulting plasmid, pAGP-3 (Figure 3) thus includes a sequence encoding the leader sequence of eco gp70, a sequence encoding mature rabbit alpha-1 acid glycoprotein; and a sequence encoding amino acid residues 263 to 469 of eco gp70.

Example 3

Plasmid pUC18RSVXeno (Figure 4), which contains the xenotropic murine leukemia virus gp70 and p15E genes under the control of an RSV promoter, was cut with AccI and StuI, and an AccI-StuI fragment encoding amino acid residues 1-258 of xeno gp70 was removed. Cloned into this site was a PCR generated AccI-StuI fragment encoding the xeno gp70 leader (amino acid residues 1-30), and the mature rabbit alpha-1 acid glycoprotein. The resulting plasmid, pAX2 (Figure 5), thus contains a sequence encoding the xeno gp70 leader, a sequence encoding the mature rabbit alpha-1 acid glycoprotein, and amino acid residues 259-443 of xeno gp70.

Example 4

Plasmid pUC18RSVXeno was cut with AccI and ClaI, and a fragment encoding amino acid residues 1-210 of xeno gp70 was removed. Cloned into this site was a PCR generated AccI-ClaI fragment encoding the xeno gp70 leader, followed by mature rabbit alpha-1 acid glycoprotein. The resulting plasmid, pAX6 (Figure 6), thus includes a sequence encoding the xeno gp70 leader, a sequence encoding mature rabbit alpha-1 acid glycoprotein, and amino acid residues 211-443 of xeno gp70.

Example 5

5×10^5 GPL cells on 10 cm tissue culture plates were transfected (using CaPO_4) with 30 $\mu\text{g}/\text{plate}$ of one of plasmids pAGP-1, pAGP-3, pAX2, or pAX6. The CaPO_4 is removed 24 hours later and 10 ml of fresh D10 medium is added for another 24 hours. The D10 medium is then removed and replaced with serum free DX medium for another 24 hours. The DX medium is then collected, filtered, and stored on ice. This supernatant contains the vector particles.

The supernatants were then filtered and collected by standard procedures and then centrifuged. After centrifugation, the virus pellets were reconstituted in a buffer containing 0.1M sodium acetate, 0.15M sodium chloride, and 2mM calcium chloride;

the buffer was sterilized using a Falcon 0.2 millimicron tissue culture filter.

2.2 ml of concentrated supernatant containing viral particles generated from pAGP-1 or pAGP-3, said viral particles sometimes hereinafter referred to as Chimeric-1 or Chimeric-3, were loaded onto two disposable plastic columns which were alcohol sterilized and dried. To each column (1cm x 6cm), one unit of neuraminidase from Clostridium perfringens which was bound to beaded agarose was added as a 2 ml suspension. This represents 1 ml of packed gel or unit of enzyme per column (15.7 mg of agarose/ml and 28 units per gram of agarose). A unit is defined as the amount of neuraminidase which will liberate 1.0 micromole of N-acetylneuraminic acid per minute from NAN-lactose at pH 5.0 and 37°C.

The columns were then washed with a large excess (50 ml) of the buffer hereinabove described to free the resin of all traces of free neuraminidase and to sterilize the resin prior to incubation with virus. The columns were then dried, and the bottoms were sealed with caps and secured with parafilm. The concentrated virus which was reconstituted in the buffer (2.0 ml per sample) was then added to the resin. The tops were placed on the columns and secured with parafilm. The resin was gently re-suspended by hand. The virus was then incubated with the resin for 1 hour at room temperature with gentle rotation on a wheel. The columns were checked periodically to ensure good mixing of resin and virus.

At the end of the incubation period, the Chimera-1 and Chimera-3 viruses were recovered by gentle vacuum filtration and collected into separate sterile 12x75 mm plastic polypropylene Falcon 2063 tubes. Recovery was greater than 90%, giving about 1.8 ml of desialated virus.

6-well plates containing about 10^5 receptor-positive (Hep G2) or receptor-negative (SK HepI) human hepatocytes in 2 ml D10 media were employed as target cells. 24 hours after the cells

were plated, 1 ml of D10 was removed from the first well and 2 ml of neuraminidase-treated (or untreated as a control) viral supernatant containing Chimeric-1 or Chimeric-3 was added and mixed well. 200 ul from the first well was diluted into the 2 ml present in the second well, was then mixed; and then 200 ul from the second well was diluted into the 1.8 ml present in the third well, thereby giving approximate dilutions of 2/3, 1/15, and 1/150. 8 ug/ml of Polybrene was included in each well during the transduction. The viral particles were left in contact with the cells overnight, followed by removal of media containing viral particles, and replaced with D10 containing 1,000 mg/ml of G418. The medium was changed with fresh D10 and G418 every 4 to 5 days as necessary. G418-resistant colonies were scored after 2 to 3 weeks.

Example 6

The pre-packaging cell line GP8, which expresses the retroviral proteins gag and pol, and the packaging cell lines derived from them which also express the chimeric gp70 glycoproteins encoded by the plasmids pAGP-1, pAGP-3, pAX2, or pAX6 were maintained in cell culture and exposed to successively higher concentrations of wheat germ agglutinin; starting with 15 ug/ml. The cell lines were maintained under WGA selection in cell culture for 6 to 8 weeks until populations resistant to 40-50 ug/ml WGA were obtained. The latter were then subjected to fluorescence-activated cell sorting using FITC-conjugated lectins to enrich for the cells expressing the desired mutant glycotype (e.g., FITC-Erythrina Cristagalli agglutinin for beta-D-galactosyl groups, and FITC-concanavalin A for alpha-D-mannosyl groups). Retroviral vector packaging and producer cell lines were then generated from the resulting populations by standard techniques.

It is to be understood, however, that the scope of the present invention is not to be limited to the specific embodiments described above. The invention may be practiced

other than as particularly described and still be within the scope of the accompanying claims.

PATAP697

SEQUENCE LISTING

(1) GENERAL INFORMATION:

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(iii) NUMBER OF SEQUENCES: 8

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(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: 3.5 inch diskette
(B) COMPUTER: IBM PS/2
(C) OPERATING SYSTEM: PC-DOS
(D) SOFTWARE: DW4.V2

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER:

(B) FILING DATE:

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Lillie, Raymond J.

(B) REGISTRATION NUMBER: 31,778

(C) REFERENCE/DOCKET NUMBER: 271010-107

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: 201-994-1700

(B) TELEFAX: 201-994-1744

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 469 bases
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

- (A) NAME/KEY: Ecotropic gp70 Protein

(x) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```

Met Ala Arg Ser Thr Leu Ser Lys Pro Leu
      5                               10
Lys Asn Lys Val Asn  Pro Arg Gly Pro Leu
      15                               20
Ile Pro Leu Ile Leu  Leu Met Leu Arg Gly
      25                               30
Val Ser Thr Ala Ser  Pro Gly Ser Ser Pro
      35                               40
His Gly Val Tyr Asn  Ile Thr Trp Glu Val
      45                               50
Thr Asn Gly Asp Arg  Glu Thr Val Trp Ala
      55                               60
Thr Ser Gly Asn His  Pro Leu Trp Thr Trp
      65                               70
Trp Pro Asp Leu Thr  Pro Asp Leu Cys Met
      75                               80
Leu Ala His His Gly  Pro Ser Tyr Trp Gly
      85                               90
Leu Glu Tyr Gln Ser  Pro Phe Ser Ser Pro
      95                               100
Pro Gly Pro Pro Cys  Cys Ser Gly Gly Ser

```

105	110
Ser Pro Gly Cys Ser	Arg Asp Cys Glu Glu
115	120
Pro Leu Thr Ser Leu	Thr Pr Arg Cys Asn
125	130
Thr Ala Trp Asn Arg	Leu Lys Leu Asp Gln
135	140
Thr Thr His Lys Ser	Asn Glu Gly Phe Tyr
145	150
Val Cys Pro Gly Pro	His Arg Pro Arg Glu
155	160
Ser Lys Ser Cys Gly	Gly Pro Asp Ser Phe
165	170
Tyr Cys Ala Tyr Trp	Gly Cys Glu Thr Thr
175	180
Gly Arg Ala Tyr Trp	Lys Pro Ser Ser Ser
185	190
Trp Asp Phe Ile Thr	Val Asn Asn Asn Leu
195	200
Thr Ser Asp Gln Ala	Val Gln Val Cys Lys
205	210
Asp Asn Lys Trp Cys	Asn Pro Leu Val Ile
215	220
Arg Phe Thr Asp Ala	Gly Arg Arg Val Thr
225	230
Ser Trp Thr Thr Gly	His Tyr Trp Gly Leu
235	240
Arg Leu Tyr Val Ser	Gly Gln Asp Pro Gly
245	250
Leu Thr Phe Gly Ile	Arg Leu Arg Tyr Gln
255	260
Asn Leu Gly Pro Arg	Val Pro Ile Gly Pro
265	270
Asn Pro Val Leu Ala	Asp Gln Gln Pro Leu

275	280
Ser Lys Pro Lys Pro	Val Lys Ser Pro Ser
285	290
Val Thr Lys Pr Pro	Ser Gly Thr Pro Leu
295	300
Ser Pro Thr Gln Leu	Pro Pro Ala Gly Thr
305	310
Glu Asn Arg Leu Leu	Asn Leu Val Asp Gly
315	320
Ala Tyr Gln Ala Leu	Asn Leu Thr Ser Pro
325	330
Asp Lys Thr Gln Glu	Cys Trp Leu Cys Leu
335	340
Val Ala Gly Pro Pro	Tyr Tyr Glu Gly Val
345	350
Ala Val Leu Gly Thr	Tyr Ser Asn His Thr
355	360
Ser Ala Pro Ala Asn	Cys Ser Val Ala Ser
365	370
Gln His Lys Leu Thr	Leu Ser Glu Val Thr
375	380
Gly Gln Gly Leu Cys	Ile Gly Ala Val Pro
385	390
Lys Thr His Gln Ala	Leu Cys Asn Thr Thr
395	400
Gln Thr Ser Ser Arg	Gly Ser Tyr Tyr Leu
405	410
Val Ala Pro Thr Gly	Thr Met Trp Ala Cys
415	420
Ser Thr Gly Leu Thr	Pro Cys Ile Ser Thr
425	430
Thr Ile Leu Asn Leu	Thr Thr Asp Tyr Cys
435	440
Val Leu Val Glu Leu	Trp Pro Arg Val Thr

445	450
Tyr His Ser Pro Ser	Tyr Val Tyr Gly Leu
455	460
Phe Glu Arg Ser Asn	Arg His Lys Arg
465	

(2) INFORMATION FOR SEQ ID NO: 2:

(I) SEQUENCE CHARACTERISTICS

(A) LENGTH: 1446 bases

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(II) MOLECULE TYPE: viral DNA

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GGCTGCCGAC CCCGGGGGTG GACCATCCTC TAGACTGACA TGGCGCGTTA AACGCTCTCA	60
AAACCCCTTA AAAATAAGGT TAACCCGCGA GGCCCCCTAA TCCCCTTAAT TCTTCTGATG	120
CTCAGAGGGG TCAGTACTGC TTCGCCCCGGC TCCAGTCCTC ATCAAGTCTA TAATATCACC	180
TGGGAGGTAA CCAATGGAGA TCGGGAGACG GTATGGGCAA CTTCTGGCAA CCACCTCTG	240
TGGACCTGGT GGCCTGACCT TACCCCAGAT TTATGTATGT TAGCCACCA TGGACCATCT	300
TATTGGGGGC TAGAATATCA ATCCCCTTTT TCTTCTCCCC CGGGGCCCCC TTGTGTCTCA	360
GGGGGCAGCA GCCCAGGCTG TTCCAGAGAC TGCGAAGAAC CTTTAACCTC CCTCACCCCT	420
CGGTGCAACA CTGCCTGGAA CAGACTCAAG CTAGACCAGA CAACTCATAA ATCAAATGAG	480
GGATTTTATG TTTGCCCCGG GCCCCACCGC CCCCAGAAT CCAAGTCATG TGGGGGTCCA	540
GA CTCCTTCT ACTGTGCTA TTGGGGCTGT GAGACAACCG GTAGAGCTTA CTGGAAGCCC	600
TCCTCATCAT GGGATTTCAT CACAGTAAAC AACAACTCTA CCTCTGACCA GGCTGTCCAG	660
GTATGCAAAG ATAATAAGTG GTGCAACCCC TTAGTTATTC GGTTTACAGA CGCCGGGAGA	720
CGGGTTACTT CCTGGACCAC AGGACATTAC TGGGGCTTAC GTTTGTATGT CTCCGGACAA	780
GATCCAGGGC TTACATTG GATCCGACTC AGATACCAA ATCTAGGACC CCGCGTCCCA	840
ATAGGGCCAA ACCCCGTTCT GGCAGACCAA CAGCCACTCT CCAAGCCCAA ACCTGTAAAG	900
TCGCCTTCAG TCACCAAACC ACCCAGTGGG ACTCCTCTCT CCCCTACCCA ACTTCCACCG	960
GCGGGAACGG AAAATAGGCT GCTAAACTTA GTAGACGGAG CCTACCAAGC CCTCAACCTC	1020
ACCAGTCCTG ACAAACCCA AGAGTGCTGG TTGTGTCTAG TAGCGGGACC CCCCTACTAC	1080

GAAGGGGTTG CCGTCCTGGG TACCTACTCC AACCATACTT CTGCTCCAGC CAACTGCTCC	1140
GTGGCCTCCC AACACAAGTT GACCCTGTCC GAAGTGACCG GACAGGGACT CTGCATAGGA	1200
GCAGTTCCCA AAACACATCA GGCCTATGT AATACCACCC AGACAAGCAG TCGAGGGTCC	1260
TATTATCTAG TTGCCCCTAC AGGTACCATG TGGGCTTGTA GTACCGGGCT TACTCCATGC	1320
ATCTCCACCA CCATACTGAA CCTTACCACT GATTATTGTG TTCTTGTCGA ACTCTGGCCA	1380
AGAGTCACCT ATCATTCCCC CAGCTATGTT TACGGCCTGT TTGAGAGATC CAACCGACAC	1440
AAAAGA	1446

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 443 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

- (A) NAME/KEY: xenotropic gp70 protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Met Glu Gly Ser Ala	Phe Ser Lys Pro Leu
5	10
Lys Asp Lys Ile Asn	Pro Trp Gly Pro Leu
15	20
Ile Val Met Gly Ile	Leu Val Arg Ala Gly
25	30
Ala Ser Val Gln Arg	Asp Ser Pro His Gln
35	40
Ile Phe Asn Val Thr	Trp Arg Val Thr Asn
45	50
Leu Met Thr Gly Gln	Thr Ala Asn Ala Thr
55	60
Ser Leu Leu Gly Thr	Met Thr Asp Thr Phe
65	70

Pro Lys Leu Tyr Phe Asp Leu Cys Asp Leu

75 80

Pro Lys Leu Tyr Phe Asp Leu Cys Asp Leu

75 80

Val Gly Asp Tyr Trp Asp Asp Pro Glu Pro

85 90

Asp Ile Gly Asp Gly Cys Arg Thr Pro Gly

95 100

Gly Arg Arg Arg Thr Arg Leu Tyr Asp Phe

105 110

Tyr Val Cys Pro Gly His Thr Val Pro Ile

115 120

Gly Cys Gly Gly Pro Gly Glu Gly Tyr Cys

125 130

Gly Lys Trp Gly Cys Glu Thr Thr Gly Gln

135 140

Ala Tyr Trp Lys Pro Ser Ser Ser Trp Asp

145 150

Leu Ile Ser Leu Lys Arg Gly Asn Thr Pro

155 160

Lys Asp Gln Gly Pro Cys Tyr Asp Ser Ser

165 170

Val Ser Ser Gly Val Gln Gly Ala Thr Pro

175 180

Gly Gly Arg Cys Asn Pro Leu Val Leu Glu

185 190

Phe Thr Asp Ala Gly	Arg Lys Ala Ser Trp
195	200
Asp Ala Pr Lys Val	Trp Gly Leu Arg Leu
205	210
Tyr Arg Ser Thr Gly	Ala Asp Pro Val Thr
215	220
Arg Phe Ser Leu Thr	Arg Gln Val Leu Asn
225	230
Val Gly Pro Arg Val	Pro Ile Gly Pro Asn
235	240
Pro Val Ile Thr Asp	Gln Leu Pro Pro Ser
245	250
Gln Pro Val Gln Ile	Met Leu Pro Arg Pro
255	260
Pro His Pro Pro Pro	Ser Gly Thr Val Ser
265	270
Met Val Pro Gly Ala	Pro Pro Pro Ser Gln
275	280
Gln Pro Gly Thr Gly	Asp Arg Leu Leu Asn
285	290
Leu Val Glu Gly Ala	Tyr Gln Ala Leu Asn
295	300
Leu Thr Ser Pro Asp	Lys Thr Gln Glu Cys
305	310
Trp Leu Cys Leu Val	Ser Gly Pro Pro Tyr
315	320

Tyr Glu Gly Val Ala	Val Leu Gly Thr Tyr
325	330
Ser Asn His Thr Ser	Ala Pro Ala Asn Cys
335	340
Ser Val Ala Ser Gln	His Lys Leu Thr Leu
345	350
Ser Glu Val Thr Gly	Gln Gly Leu Cys Val
355	360
Gly Ala Val Pro Lys	Thr His Gln Ala Leu
365	370
Cys Asn Thr Thr Gln	Lys Thr Ser Asp Gly
375	380
Ser Tyr Tyr Leu Ala	Ala Pro Ala Gly Thr
385	390
Ile Trp Ala Cys Asn	Thr Gly Leu Thr Pro
395	400
Cys Leu Ser Thr Thr	Val Leu Asn Leu Thr
405	410
Thr Asp Tyr Cys Val	Leu Val Glu Leu Trp
415	420
Pro Lys Val Thr Tyr	His Ser Pro Asp Tyr
425	430
Val Tyr Gly Gln Phe	Glu Lys Lys Thr Lys
435	440
Tyr Lys Arg	

(2) INFORMATION FOR SEQ ID NO: 4:

(I) SEQUENCE CHARACTERISTICS

(A) LENGTH: 1356 bases

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(II) MOLECULE TYPE: viral DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GCGACAATC CTCCAGCCGG GAACAGCATG GAAGGTTGAG CGTTCTCAA ACCCCTTAAA	60
GATAAGATTA ACCCGTGGGG CCCCTAATA GTTATGGGGA TCTTGGTGAG GGCAGGAGCT	120
TCGGTACAAC GTGACAGCCC TCACCAGATC TTCAATGTTA CTTGGAGAGT TACCAACCTA	180
ATGACAGGAC AAACAGCTAA CGCCACCTCC CTCCTGGGGA CGATGACAGA CACCTTCCCT	240
AACTATATT TTGACCTGTG TGATTTAGTA GGAGACTACT GGGATGACCC AGAACCCGAT	300
ATTGGGGATG GTTGCCGCAC TCCCGGGGGA AGAAGAAGGA CAAGACTGTA TGA CTCTCTAT	360
GTTTGCCCCG GTCATACTGT ACCAATAGGG TGTGGAGGGC CGGGAGAGGG CTACTGTGGC	420
AAATGGGGAT GTGAGACCAC TGGACAGGCA TACTGGAAGC CATCATCATC ATGGGACCTA	480
ATTTCCCTTA AGCGAGGAAA CACTCCTAAG GATCAGGGCC CCTGTTATGA TTCCTCGGTC	540
TCCAGTGGCG TCCAGGGTGC CACACCGGGG GGTCGATGCA ACCCCCTGGT CTTAGAATTC	600
ACTGACGCGG GTAGAAAGGC CAGCTGGGAT GCCCCCAAAG TTTGGGGACT AAGACTCTAT	660
CGATCCACAG GGGCCGACCC GGTGACCCGG TTCTCTTTGA CCCGCCAGGT CCTCAATGTA	720
GGACCCCGCG TCCCCATTGG GCCTAATCCC GTGATCACTG ACCAGCTACC CCCATCCCAA	780
CCCGTGCAGA TCATGCTCCC CAGGCCTCCT CATCCTCCTC CTTCAGGCAC GGTCTCTATG	840
GTACCTGGGG CTCCCCCGCC TTCTCAACAA CCTGGGACGG GAGACAGGCT GCTAAATCTG	900
GTAGAAGGAG CCTACCAAGC ACTCAACCTC ACCAGTCCTG ACAAACCCA AGAGTGCTGG	960
TTGTGTCTGG TATCGGGACC CCCCTACTAC GAAGGGCTTG CCGTCCTAGG TACCTACTCC	1020

AACCATACCT CTGCCCCAGC TAACTGCTCC GTGGCCTCCC AACACAAGCT GACCCTGTCC	1080
GAAGTAACCG GACAGGGACT CTGCGTAGGA GCAGTTCCCA AAACCCATCA GGCCCTGTGT	1140
AATACCACCC AGAAGACGAG CGACGGGTCC TACTATCTGG CTGCTCCCGC CGGGACCATC	1200
TGGGCTTGCA ACACCGGGCT CACTCCCTGC CTATCTACTA CTGTACTCAA CCTCACCACC	1260
GATTACTGTG TCCTGGTTGA GCTCTGGCCA AAGGTAACCT ACCACTCCCC TGATTATGTT	1320
TATGGCCAGT TTGAAAAGAA AACTAAATAT AAAAGA	1356

(2) INFORMATION FOR SEQ ID NO: 5:

(I) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 201 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(II) MOLECULE TYPE: protein

(ix) FEATURE

- (A) NAME/KEY: rabbit alpha-1-acid glycoprotein

(x) PUBLICATION INFORMATION

- (A) AUTHOR Ray, et al.
(B) TITLE:
(C) JOURNAL: Biochem. and Biophys. Res. Comm.
(D) VOLUME: 178
(E) ISSUE: No. 2
(F) PAGES: 507-513
(G) DATE: 1991

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Met Ala Leu Pro Trp	Ala Leu Ala Val Leu
5	10
Ser Leu Leu Pro Leu	Leu His Ala Gln Asp
15	20
Pro Ala Cys Ala Asn	Phe Ser Thr Ser Pro
25	30

Ile Thr Asn Ala Thr	Leu Asp Gln Leu Ser
35	40
His Lys Trp Phe Phe	Thr Ala Ser Ala Phe
45	50
Arg Asn Pro Lys Tyr	Lys Gln Leu Val Gln
55	60
His Thr Gln Ala Ala	Phe Phe Tyr Phe Thr
65	70
Ala Ile Lys Glu Glu	Asp Thr Leu Leu Leu
75	80
Arg Glu Tyr Ile Thr	Thr Asn Asn Thr Cys
85	90
Phe Tyr Asn Ser Ser	Ile Val Arg Val Gln
95	100
Arg Glu Asn Gly Thr	Leu Ser Lys His Asp
105	110
Gly Ile Arg Asn Ser	Val Ala Asp Leu Leu
115	120
Leu Leu Arg Asp Pro	Gly Ser Phe Leu Leu
125	130
Val Phe Phe Ala Gly	Lys Glu Gln Asp Lys
135	140
Gly Met Ser Leu Tyr	Thr Asp Lys Pro Lys
145	150
Ala Ser Thr Glu Gln	Leu Glu Glu Phe Tyr
155	160

Glu Ala Leu Thr Cys	Leu Gly Met Asn Lys
165	170
Thr Glu Val Val Tyr	Thr Asp Trp Thr Lys
175	180
Asp Leu Cys Glu Pro	Leu Glu Lys Gln His
185	190
Glu Glu Glu Arg Lys	Lys Glu Lys Ala Glu
195	200
Ser	

(2) INFORMATION FOR SEQ ID NO: 6

(1) SEQUENCE CHARACTERISTICS

(A) LENGTH: 759 bases

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(H) MOLECULE TYPE: genomic DNA

(x) PUBLICATION INFORMATION:

(A) AUTHOR Ray, et al.

(B) TITLE:

(C) JOURNAL: Biochem. and Biophys. Res. Comm.

(D) VOLUME: 178

(E) ISSUE: NO. 2

(F) PAGES: 507-513

(G) DATE: 1991

(x) SEQUENCE DESCRIPTION: SEQ ID NO:6:

AGCTCTGCCT GGCTCCAGCG CCTCTGTGTC TCAGCATGGC CCTGCCCTGG GCCCTCGCCG	60
TCCTGAGCCT CTTCCCTCTG CTGCATGCCC AGGACCCAGC GTGTGCCAAC TTCTCGACCA	120
GCCCTATCAC CAATGCCACC CTGGACCAGC TCTCCACAA GTGGTTTTTT ACCGCCTCGG	180
CCTTCCGGAA CCCCAAGTAC AAGCAGCTGG TGCAGCATAC CCAGGCGGCC TTTTCTACT	240
TCACCGCCAT CAAAGAGGAG GACACCTTGC TGCTCCGGGA GTACATAACC ACGAACAACA	300
CGTGCTTCTA TAACTGCAGC ATCGTGAGGG TCCAGAGAGA GAATGGGACC CTCTCCAAAC	360
ACGACGGCAT ACGAAATAGC GTGGCCGACC TGCTGCTCCT CAGGGACCCC GGGAGCTTCC	420
TCCTCGTCTT CTTGCTGGG AAGGAGCAGG ACAAGGGAAT GTCCTTCTAC ACCGACAAGC	480
CCAAGGCCAG CCCGGAACAA CTGGAAGAGT TCTACGAAGC CCTCACGTGC CTGGGCATGA	540

```

ACAAGACGGA AGTCGTCTAC ACTGACTGGA CAAAGGATCT GTGCGAGCCG CTGGAGAAGC      600
AACACGAGGA GGAGAGGAAG AAGGAAAAGG CAGAGTCATA GGGCACAGCA CCGGCTCCGG      660
GACTCGGGGC CCACCCCTG CACCTGCCTT TTTGTTTGTT TTGTAAATCT CTGTTCTTTC      720
CCATGGTTGC ATCAATAAAA CTGCTGGACC AGTAAAAAA      759

```

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 196 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

(A) NAME/KEY: ecotropic p15E protein.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

```

Glu Pro Val Ser Leu Thr Leu Ala Leu Leu
      5              10
Leu Gly Gly Leu Thr Met Gly Gly Ile Ala
      15              20
Ala Gly Ile Gly Thr Gly Thr Thr Ala Leu
      25              30
Met Ala Thr Gln Gln Phe Gln Gln Leu Gln
      35              40
Ala Ala Val Gln Asp Asp Leu Arg Glu Val
      45              50

```

Glu Lys Ser Ile Ser Asn Leu Glu Lys Ser	
55	60
Leu Thr Ser Leu Ser Glu Val Val Leu Gln	
65	70
Asn Arg Arg Gly Leu Asp Leu Leu Phe Leu	
75	80
Lys Glu Gly Gly Leu Cys Ala Ala Leu Lys	
85	90
Glu Glu Cys Cys Phe Tyr Ala Asp His Thr	
95	100
Gly Leu Val Arg Asp Ser Met Ala Lys Leu	
105	110
Arg Glu Arg Leu Asn Gln Arg Gln Lys Leu	
115	120
Phe Glu Ser Thr Gln Gly Trp Phe Glu Gly	
125	130
Leu Phe Asn Arg Ser Pro Trp Phe Thr Thr	
135	140
Leu Ile Ser Thr Ile Met Gly Pro Leu Ile	
145	150
Val Leu Leu Met Ile Leu Leu Phe Gly Pro	
155	160
Cys Ile Leu Asn Arg Leu Val Gln Phe Val	
165	170
Lys Asp Arg Ile Ser Val Val Gln Ala Leu	
175	180

Val Leu Thr Gln Gln Tyr His Gln Leu Lys

185

190

Pro Ile Glu Tyr Glu Pro

195

INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 176 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

(A) NAME/KEY: HTLV-I p21 protein

(x) PUBLICATION INFORMATION:

(A) AUTHOR: Malik, et al.

(B) TITLE:

(C) JOURNAL: J. Gen. Virol.

(D) VOLUME: 69

(E) ISSUE:

(F) PAGES: 1695-1710

(G) DATE: 1988

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Ala Val Pro Val Ala Val Trp Leu Val Ser

5 10

Ala Leu Ala Met Gly Ala Gly Val Ala Gly

15 20

Arg Ile Thr Gly Ser Met Ser Leu Ala Ser

25 30

Gly Lys Ser Leu Leu His Glu Val Asp Lys

35 40

Asp Ile Ser Gln Leu Thr Gln Ala Ile Val

45 50

Lys Asn His Lys Asn Leu Leu Lys Ile Ala	
55	60
Gln Tyr Ala Ala Gln Asn Arg Arg Gly Leu	
65	70
Asp Leu Leu Phe Trp Glu Gln Gly Gly Leu	
75	80
Cys Lys Ala Leu Gln Glu Gln Cys Cys Phe	
85	90
Leu Asn Ile Thr Asn Ser His Val Ser Ile	
95	100
Leu Gln Glu Arg Pro Pro Leu Glu Asn Arg	
105	110
Val Leu Thr Gly Trp Gly Leu Asn Trp Asp	
115	120
Leu Gly Leu Ser Gln Trp Ala Arg Glu Ala	
125	130
Leu Gln Thr Gly Ile Thr Leu Val Ala Leu	
135	140
Leu Leu Leu Val Ile Leu Ala Gly Pro Cys	
145	150
Ile Leu Arg Gln Leu Arg His Leu Pro Ser	
155	160
Arg Val Arg Tyr Pro His Tyr Ser Leu Ile	
165	170
Asn Pro Glu Ser Ser Leu	
175	

WHAT IS CLAIMED IS:

1. A retroviral vector particle, said vector particle including a receptor binding region that binds to a receptor of a target cell, said receptor of a target cell being other than the amphotropic cell receptor.
2. The vector particle of Claim 1 wherein said vector particle is a murine leukemia virus particle.
3. The vector particle of Claim 2 wherein said vector particle includes gp70 protein, and wherein a portion but not all of the gp70 protein has been deleted and replaced with said receptor binding region that binds to a receptor of a target cell.
4. The vector particle of Claim 1 wherein said receptor binding region is a receptor binding region of a human virus.
5. The vector particle of Claim 4 wherein said receptor binding region of a human virus is a hepatitis B virus surface protein binding region and said target cell is a liver cell.
6. The vector particle of Claim 4 wherein said receptor binding region of a human virus is the receptor binding region of gp46 of HTLV-I virus, and said target cell is a T-cell.
7. The vector particle of Claim 4 wherein said receptor binding region of a human virus is the HIV gp120 CD4 binding region and said target cell is a T4 helper cell.
8. The vector particle of Claim 2 wherein said vector particle contains a chimeric protein encoded by DNA (RNA) wherein at least a portion of the DNA (RNA) encoding the receptor binding domain of gp70 protein is removed and is replaced with DNA (RNA) which encodes a protein which binds to an asialoglycoprotein receptor of hepatocytes.

9. The vector particle of Claim 8 wherein said protein which binds to an asialoglycoprotein receptor of hepatocytes is alpha-1 acid glycoprotein.

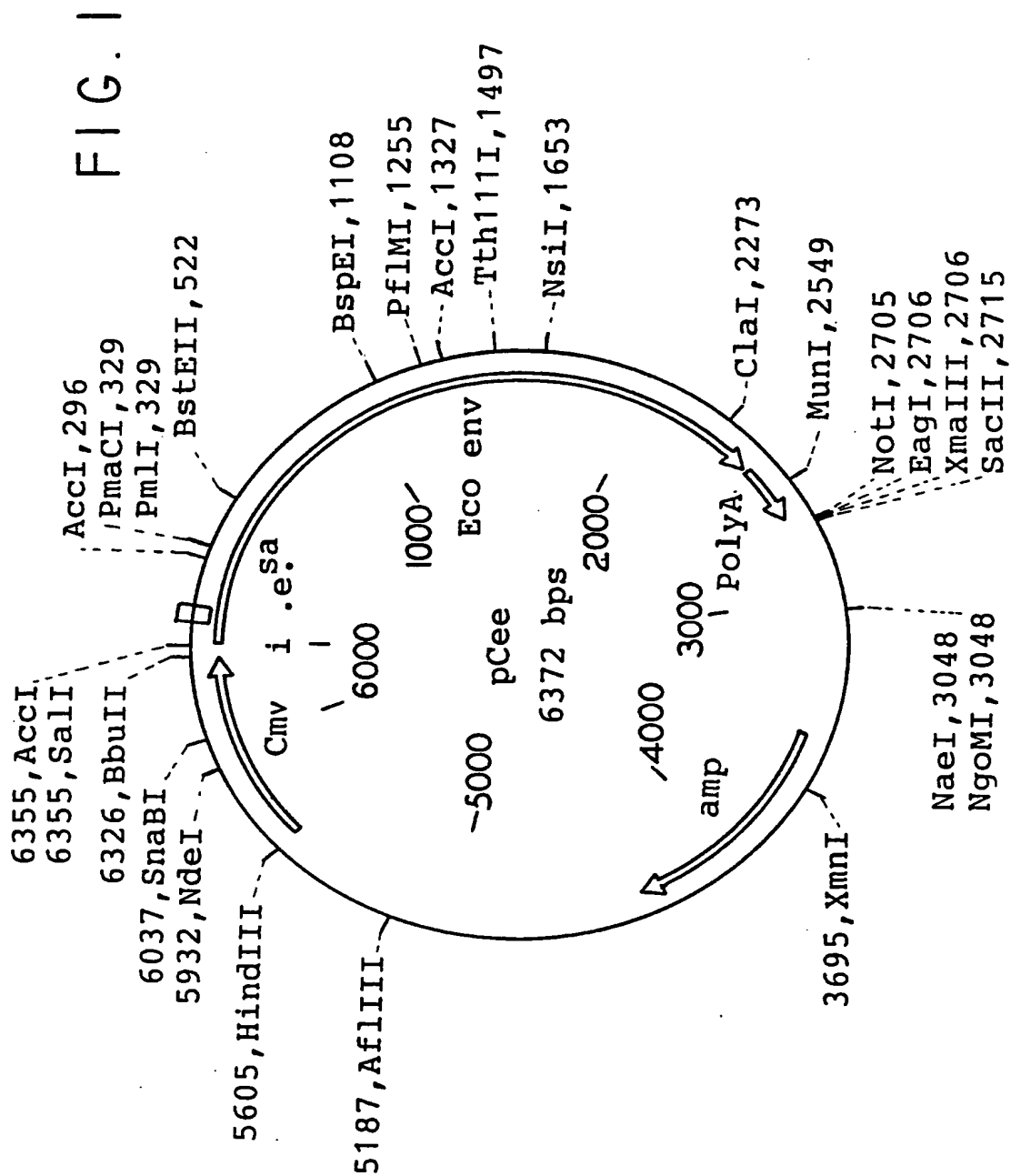
10. The vector particle of Claim 1 and further including at least one heterologous gene.

11. A method of introducing at least one heterologous gene into a target cell, comprising, administering to said target cell the vector particles of Claim 10.

12. The method of Claim 11 wherein said vector particles are administered ex vivo.

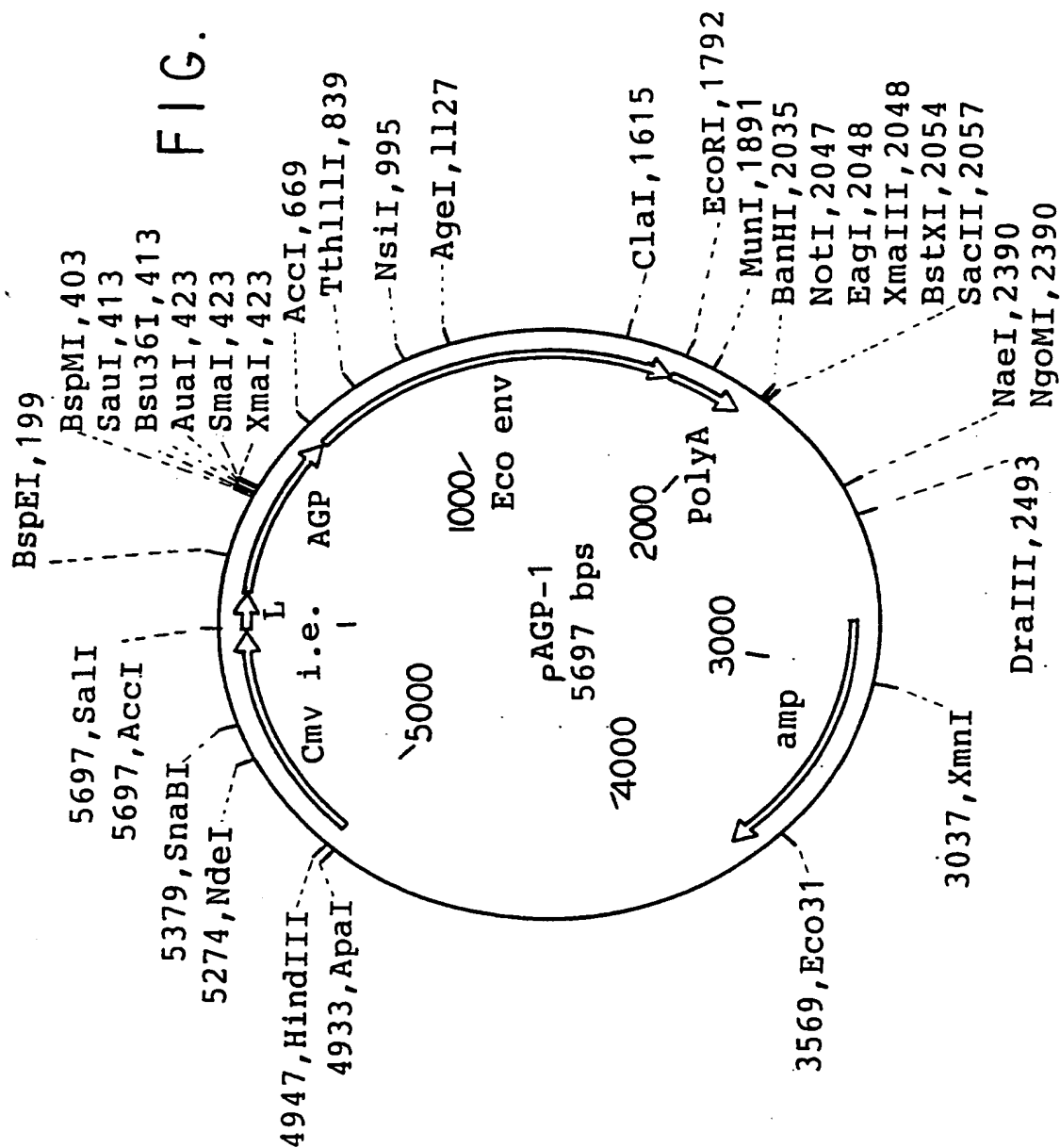
13. The method of Claim 11 wherein said vector particles are administered in vivo.

14. A packaging cell line which produces the retroviral particles of Claim 1.



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FIG. 2



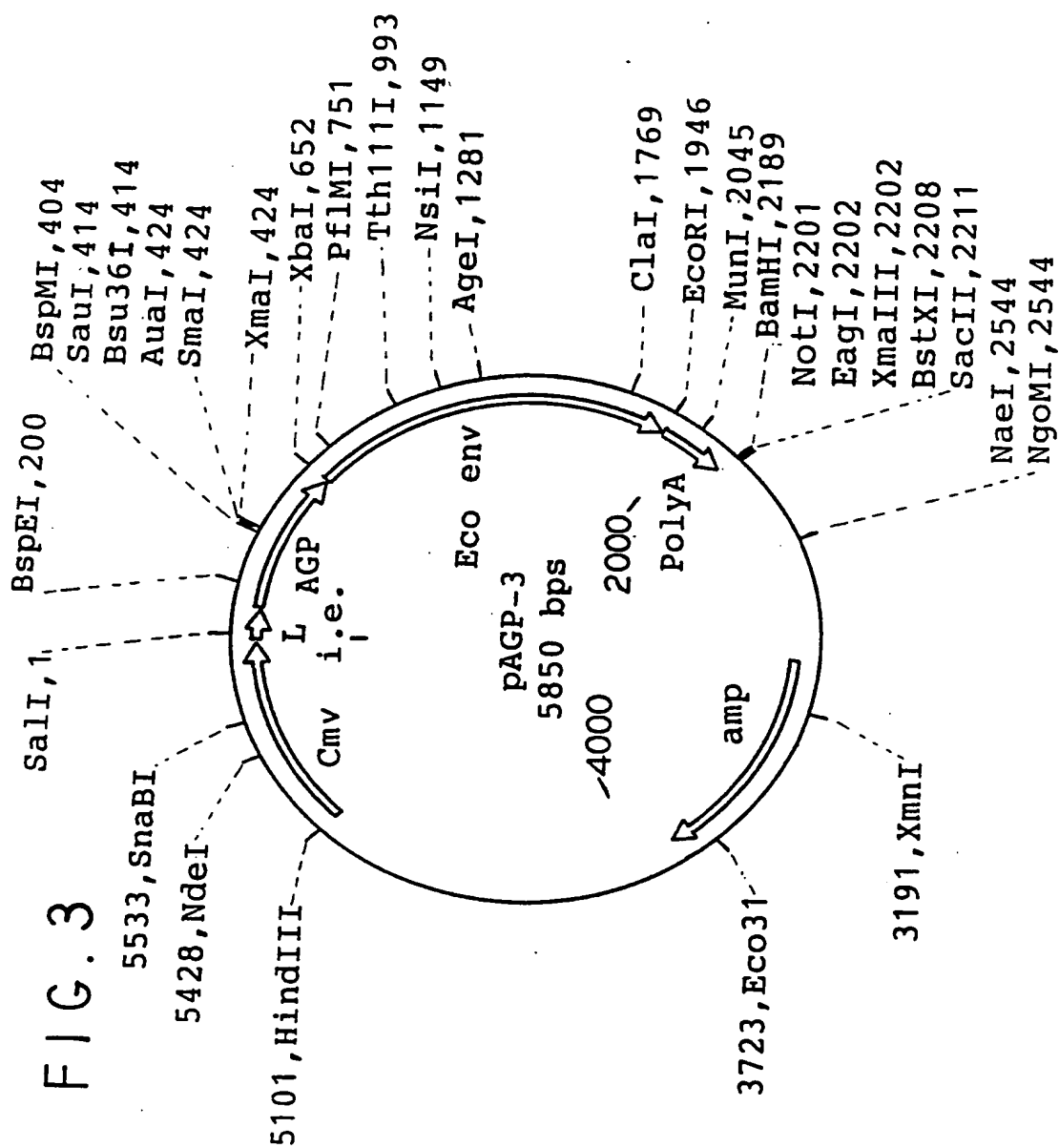
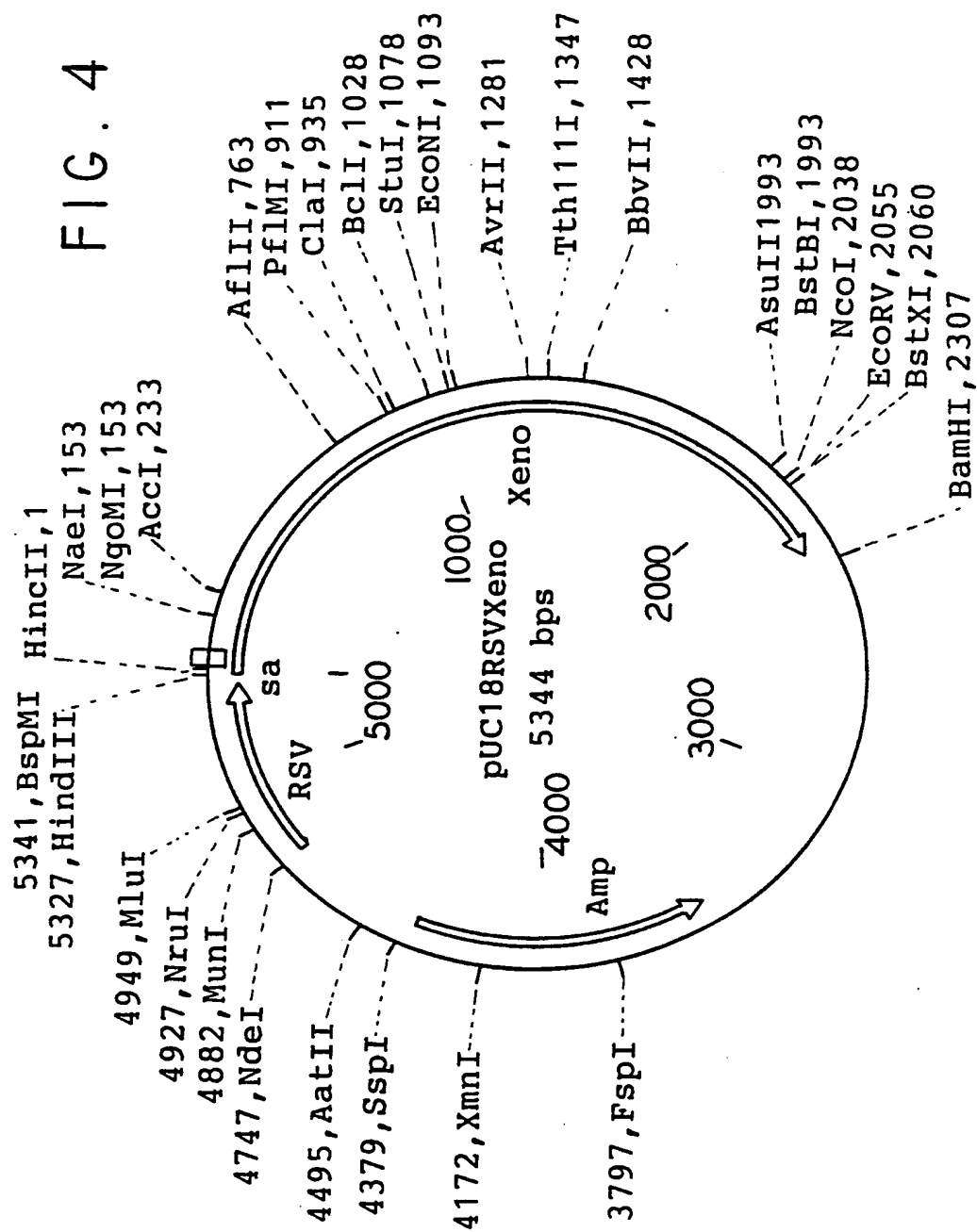
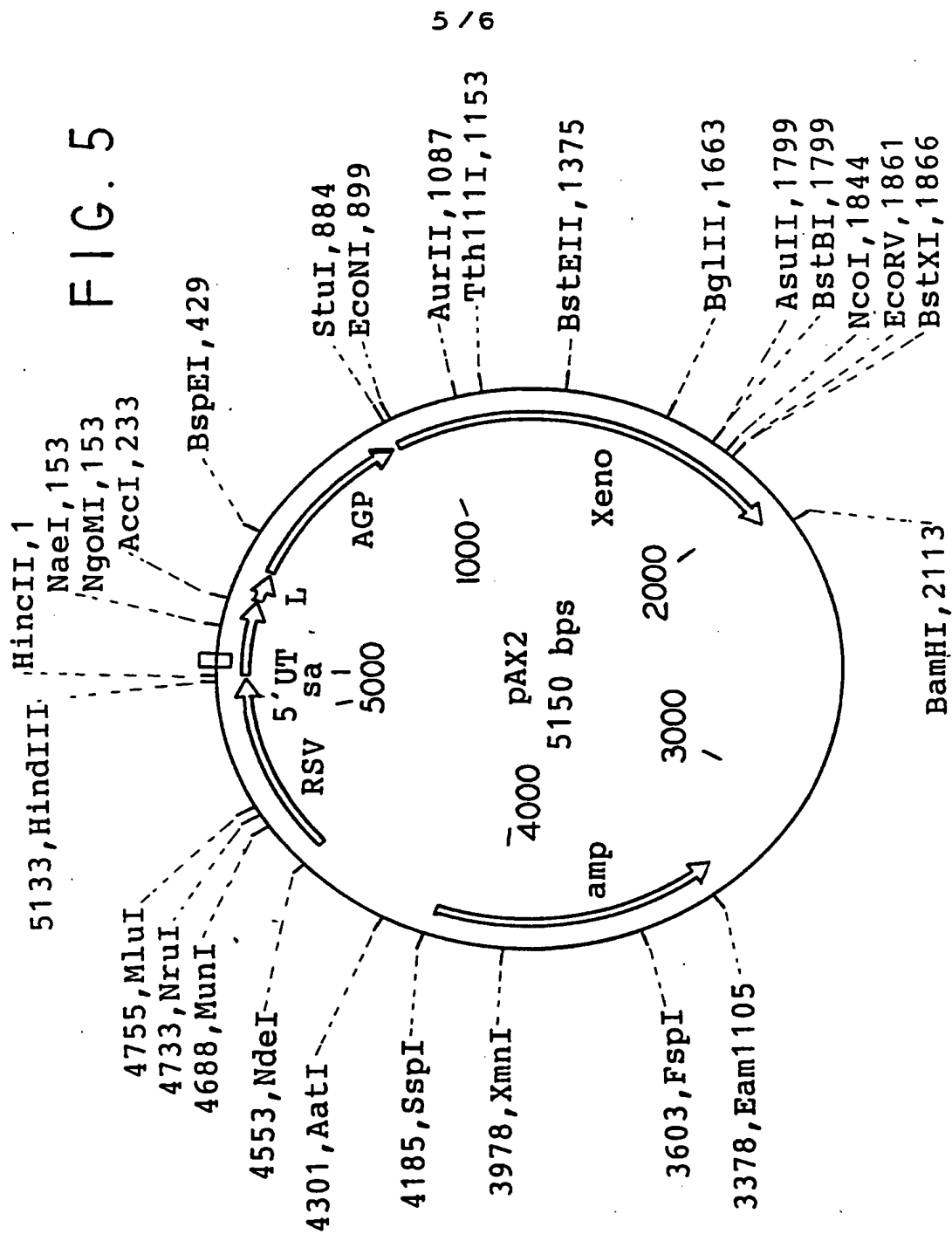


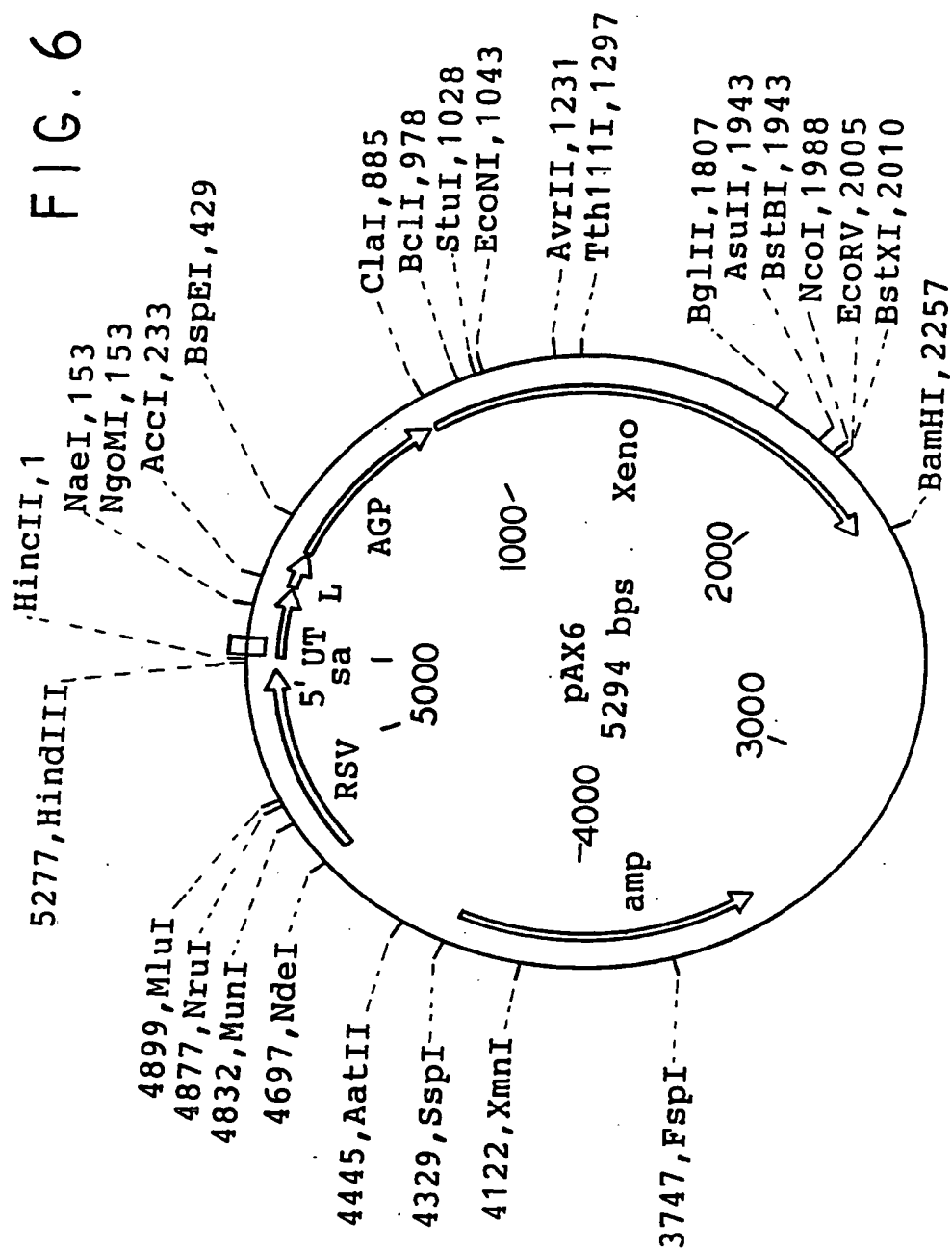
FIG. 4





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FIG. 6



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US93/10522

A. CLASSIFICATION OF SUBJECT MATTER																										
IPC(5) : C12P 21/00; C12N 15/00, 15/58, 15/40, 15/48, 15/63, 15/86 US CL : 435/320.1, 69.1, 240.2; 424/93; 935/23, 32, 52, 57, 66, 70 According to International Patent Classification (IPC) or to both national classification and IPC																										
B. FIELDS SEARCHED																										
Minimum documentation searched (classification system followed by classification symbols) U.S. : 435/320.1, 69.1, 240.2; 424/93; 935/23, 32, 52, 57, 66, 70																										
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched																										
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) APS, Dialog, Biosis, Biotech, Medicine, Medline Search Terms: retrovirus, vector, receptor, receptor binding protein																										
C. DOCUMENTS CONSIDERED TO BE RELEVANT																										
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.																								
A	Journal of Virology, Volume 61, No. 5, issued May 1987, M.A. Bender et al., "Evidence that the Packaging Signal of Moloney Murine Leukemia Virus Extends Into the gag Region" pages 1639-1646, See particularly page 1640.	1-14																								
A	Biotechniques, Volume 7, No. 9, issued 1989, A.D. Miller et al., "Improved Retroviral Vectors for Gene Transfer and Expression" pages 980-990, See particularly page 984.	1-14																								
<input type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.																										
<table border="0"> <tr> <td colspan="2">* Special categories of cited documents:</td> <td>*T</td> <td>later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</td> </tr> <tr> <td>*A</td> <td>document defining the general state of the art which is not considered to be part of particular relevance</td> <td>*X</td> <td>document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</td> </tr> <tr> <td>*E</td> <td>earlier document published on or after the international filing date</td> <td>*Y</td> <td>document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</td> </tr> <tr> <td>*L</td> <td>document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</td> <td>*Z</td> <td>document member of the same patent family</td> </tr> <tr> <td>*O</td> <td>document referring to an oral disclosure, use, exhibition or other means</td> <td></td> <td></td> </tr> <tr> <td>*P</td> <td>document published prior to the international filing date but later than the priority date claimed</td> <td></td> <td></td> </tr> </table>			* Special categories of cited documents:		*T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention	*A	document defining the general state of the art which is not considered to be part of particular relevance	*X	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone	*E	earlier document published on or after the international filing date	*Y	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art	*L	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Z	document member of the same patent family	*O	document referring to an oral disclosure, use, exhibition or other means			*P	document published prior to the international filing date but later than the priority date claimed		
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Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE		Authorized officer DAVID GUZO <i>D. Guzo</i> Telephone No. (703) 308-0196																								